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METHOD FOR DIAGNOSING PHEOCHROMOCYTOMA

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ABSTRACT OF THE DISCLOSURE

A new, accurate method for disgnosing pheochromocytoma which comprises determining the pressor response in systolic blood pressure to intravenous injection of tyramine. The baseline systolic blood pressure of a patient is determined, an intravenous injection of 250 to 1000 micrograms of tyramine is administered, and a rise in systolic blood pressure greater than 20 mm. of mercury is observed if pheochromocytoma is present.

This invention relates to a new method of diagnosis. More specifically, this invention relates to a new and more accurate method for diagnosing pheochromocytoma which comprises determining the pressor response in systolic blood pressure to an intravenous injection of 250 to 1000 micrograms of a pharmaceutically acceptable salt of tyramine (4-hydroxyphenethylamine) based on the free base whereby a pressor response greater than 20 mm. of mercury in systolic blood pressure shows the presence of said disease. This invention also relates to compositions for such use.

Pheochromocytoma is defined as a catecholamine-producing tumor of the adrenal medulla and other tissues. Its tissue contains the catecholamine hormones, epinephrine, adrenaline or norepinephrine (noradrenaline) or both and its presence is associated with many signs and symptoms, particularly paroxysmal hypertension. Other terms such as chromaffinoma and paraganglioma, have been used in the past but pheochromocytoma is the preferred designation and refers to a hormonally-active tumor.

Although measurements of catecholamines and their metabolites in the urine provide the most reliable means of diagnosing pheochromocytoma, the specialized nature of the procedures involved are such that reliable measurements are unavailable to many physicians. Thus, considerable reliance must still be placed on pharmacologic aids to diagnosis. These are of two types: vesopressor provocative tests and the vasodepressor blocking tests. Though several different compounds have been used in such tests, only the original provocative agent, histamine and the blocking test employing phenotolamine have retained favor among clinicians. The morbidity associated with intravenous injection of histamine is well known. Flushing and severe throbbing headache, while transient, are very disturbing to the patient, and the use of this agent is contraindicated in aged patients and those with coronary disease or asthma. In the presence of pheochromocytoma alarming pressor effects and severe morbidity may be encountered with the use of histamine, and at least two deaths have been reported. For these reasons, some physicians do histamine tests reluctantly, or not at all.

We have found a new and safe and more effective method for the diagnosis of pheochromocytoma, a method which is effective even when older methods give false results.

The object of the present invention is a safe and reliable provocative agent whose use is not attended by significant morbidity or hazard. A theoretical basis for such a

test seems to exist if one assumes that increased tissue stores of catecholamines occur at sympathetic nerve endings in patients with pheochromocytoma as a result of uptake from the blood. If this is so, an exaggerated pressor response might be expected following injection of a so-called indirectly-acting sympathomimetic compound, i.e., one whose effects are mediated by release of norepinephrine at nerve endings. The compound of this type which we have found useful is tyramine. It is the essence of this invention that we find tyramine offers the advantage of a more predictable pressor response and an absence of undesirable side effects.

In the method of this invention, tyramine hydrochloride is conveniently prepared in a concentration of 10 mg tyramine base (12.66 mg. tyramine hydrochloride) per ml. water with 0.1% sodium metabisulfite as preservative and sterilized by filtration by Dr. Joseph Gillelli, Pharmaceutical Development Service of the National Institutes of Health. This preparation is stable for at least one year when kept in sealed dark bottles at 4° C. Just prior to use, the tyramine is diluted to a concentration of 200 to 1000 μg. per ml. with sterile isotonic saline. Other pharmaceutically acceptable salts of tyramine such as the sulfate, nitrate, phosphate, citrate, hydrobromide and the like can equally well be used. Similarly, in the preparation of the compositions for use in this invention, other pharmaceutically acceptable preservatives, benzyl-alcohol, phenol, chlorobutanol or a mixture of the methyl and propyl esters of p-hydroxybenzoic acid, may be used.

The compositions for use in this invention comprise a sterile isotonic aqueous solution of a pharmaceutically acceptable salt of tyramine, the concentration of tyramine base being of the order of 250 to 1000 micrograms per milliliter. Ordinarily, and preferably, it is a 1 cc. or 2 cc. unit dose of 1000 μg./ml. concentration. Thus, a 1 cc. vial contains just enough for one 1.0 mg. injection. This is contemplated as the standard one injection test dose. If a series of doses are to be used, the 2 cc. vial of 1 mg./ml. concentration permits a 250 μg., a 500 μg., and a 1 mg. dose to be given successively by successive withdrawal of ¼, ½ and 1 cc. from a 2 cc. vial.

The response to tyramine injection is studied with the subject resting in a quiet room. An intravenous infusion of 5 percent dextrose in water is started in one arm, and indirect blood pressures using a mercury manometer and inflatable cuff are determined in the other arm. The blood pressure is measured every two minutes until it stablilizes (usually 10–20 minutes), and then single injections of placebo (isotonic sodium chloride solution) or graded doses of tyramine are given through a three-way stopcock at the proximal end of the infusion tubing, followed by rapid flushing of the material into the patient with 5–10 ml. of 5 percent of dextrose in water. The blood pressure is recorded every thirty seconds after injection until the systolic pressure returns to baseline. The blood pressure may then be recorded every minute for an additional 10–15 minutes after which another injection may be given.

Before doses of tyramine are given to the subjects, it is desirable to establish that there is no response to the injection of the placebo. This usually requires no more than one or two placebo injections. Once this is achieved, increasing doses of tyramine are given, the initial dose being 250 μg. The usual doses employed are 250, 500, 1000, 1500 and 2000 μg. with higher doses given in 1000 μg. increments to a limit of 6000 μg. as permitted by the response in the blood pressure. Because of their increased pressor responsiveness to tyramine, four of five patients tested with pheochromocytoma were given no more than 1000 μg. and the fifth patient received a maximum dose of only 1500 μg.

When a minimally effective pressor dose is established in each subject, varying doses are given in randomized fashion to achieve reproducible responses. Dose response curves are constructed for each subject using the average of at least two peak systolic pressor responses at each level of dosage. Changes in diastolic blood pressure are inconsistent and irrelevant.

In the present study a systolic blood pressure rise of greater than 20 mm. Hg following intravenous injection of 1000 μg. or less of tyramine base occurs only in cases of pheochromocytoma. Specificity is indicated by the lesser responses to tyramine in hypertensive and normotensive subjects without pheochromocytoma and the conversion of tyramine pressor responses to normal in two patients by removal of their tumors. Furthermore, the tyramine responsiveness is shown to be normal in four hypertensive patients who had borderline or false positive histamine tests.

When tyramine has been administered by this protocol outlined, there have been no serious pressor episodes, and only minimal discomfort in the form of palpitations was experienced by a few of the patients. In all cases that have a pressor response of greater than 20 mm. Hg to this dose of tyramine, further evaluation of the diagnosis of pheochromocytoma should be pursued by specific chemical analysis of the urine for the catecholamines or their metabolites.

This invention can be illustrated by the following detailed example of its use and the examples of compositions to be used.

Example 1

Three groups of hospitalized subjects were studied: (1) five female patients, ages 12 to 71 years, with pheochromocytoma, (2) 16 male and five female patients, ages 16 to 57 years, with essential hypertension of varying severity, and (3) five male and six female healthy volunteers, ages 17 to 43 years, with normal blood pressure.

As shown in Table I, the patients with pheochromocytoma had blood pressure levels in the range of 118/80 mm. Hg to 168/122 mm. Hg at the time of testing with tyramine. It may also be seen that the urinary excretions of the catecholamines and their metabolites were diagnostic of pheochromocytoma in each case. Patients 1 and 2 had their primary tumors resected, but at the time of this study, they had histologically proven malignant pheochromocytoma with widespread metastases. Patients 3 and 4 had benign tumors which were subsequently removed with apparent resultant cure. The fifth patient, 5, is presumed to have a benign tumor, but surgical exploration has been deferred because of medical contraindications. No patient with pheochromocytoma was receiving anti-hypertensive medications at the time of our study, although large doses of intramuscular reserpine were discontineud two days before testing in patient 2. Patients 1, 2 and 5 have each been managed successfully for longer than a year by oral administration of phenoxybenzamine.

The patients with essential hypertension separated into two groups on the basis of their control blood pressures at the time of the studies: (a) "mild" hypertension; 12 patients with untreated labile or sustained hypertension in the range of 140 to 200 mm. Hg systolic and less than 100 mm. Hg diastolic, and (b) severe hypertension; nine patients with untreated sustained hypertension greater than 200/110 mm. Hg.

The data obtained by these tests are summarized in the series of tables which follow in which Table I shows the catecholamine data on five patients with the disease and Table II shows the normal tyramine responses in hypertensive patients with borderline or false histamine tests. Table III shows the typical response to tyramine from a patient with pheochromocytoma. Table IV shows the ranges of response and the mean responses of groups of individuals in various categories, including normal persons, hypertensives and those with pheochromocytoma. In Table V is summarized the dose response curves of these categories and the detailed dose response of persons with the tumor while Table VI gives the dose response of individuals before and after surgical excision of the tumor.

TABLE I.— DATA IN FIVE PATIENTS WITH PHEOCHROMOCYTOMA

Patient (age, sex)	Control Blood Pressure[1] (mm. Hg)	NE (μg./day)	E (μg./day)	NMN+MN (mg./day)	VMA (mg./day)
1 (12, F)	167/118	524	47	3.5	15.6
2 (12, F)	155/108	3,140	N.S.	16.9	52.2
3 (26, F)	168/122, [2] (115/80)	1,960	N.S.	6	20.7
4 (38, F)	118/80, [2] (110/80)	2,700	1,800	122	600
5 (71, F)	120/68	72	625	19.8	40
Upper Limit of Normal		80	20	1.3	6.0

[1] Average control blood pressure during the pretreatment tyramine response tests.
[2] Numbers in parenthesis represent average control blood pressures during post-operative tyramine response tests.
NOTE.—NE=unconjugated norepinephrine, E=unconjugated epinephrine, NMN+MN=normetanephrine+matanephrine, VMA=vanilmandelic acid, N.S.=not significant; significant value for E cannot be determined when it constitutes less than 5% of the total daily catecholamine excretion.

TABLE II.—NORMAL TYRAMINE RESPONSES IN HYPERTENSIVE PATIENTS WITH BORDERLINE OR FALSE POSITIVE HISTAMINE TESTS

Patient (age, sex)	Systolic and Diastolic Maximum Rise in Blood Pressure (mm. Hg)					Urinary Catecholamine Excretion (μg./hr.) (normal 4.0)	
	Histamine Dose*		Tyramine Dose*		Cold Pressor Test	Control	After Histamine, 2 hr. sample
	25 μg.	50 μg.	500 μg.	1,000 μg.			
False Positive:							
6 (48, M)	60/30	84/30	0/0	5/0	35/15	2.0	2.0
7 (47, M)	80/38	----------	0/0	0/0	56/28	2.0	3.1
Borderline Positive:							
8 (14, M)	20/20	56/30	0/0	6/0	6/0	----------	1.1
9 (6, M)	30/30	58/25	0/0	10/0	35/42	1.1	0.9

*Expressed as free base of compound.

TABLE III.—TYPICAL RESPONSE TO TYRAMINE FOR PATIENT WITH PHEOCHROMOCYTOMA (PATIENT NO. 2)

Time from 600 μg Tyramine injection, min.	Blood pressure (systolic), mm. Hg
−5	140
−4	142
−3	140
−2	146
−1	140
0	139
1	194
2	198
3	190
4	189
5	180
6	165
7	160
8	148
10	142
12	142

TABLE IV.—PRESSOR RESPONSES FOR INDIVIDUALS IN EACH GROUP TO 1,000 μg. TYRAMINE

Group	Change in Blood Pressure, mm. Hg	
	Range	Mean
(1) Pheochromocytoma (5 patients)	20–62	42
(2) Mild Hypertensives (12 patients)	0–15	4.5
(3) Severe Hypertensives (9 patients)	0–13	7.0
(4) Normal Persons (11 subjects)	0–8	2.8

TABLE V.—DOSE RESPONSE IN MM. Hg BLOOD PRESSURE INCREASE

	Dose, μg. Tyramine Base			
	500	1,000	1,500	2,000
Patients:				
1	43	62		
2	40	50		
3	18	34		
4	29	45		
5	16	20	40	
Hypertensives without Pheochromocytoma	2.5	6	11.5	14.7
Average of 21 patients (Range)	(0–10)	(0–15)	(0–24)	(4–32)
Normal: Average of 11 persons	1.3	2.8	6.7	8.8
Range	(0–5)	(0–8)	(0–16)	(0–22)

TABLE VI.—COMPARISON OF DOSE RESPONSE BEFORE AND AFTER SURGICAL REMOVAL OF TUMOR

[Expressed in mm. Hg of increase in systolic pressure]

Tyramine (μg.)	Patient No. 1		Patient No. 2	
	Before	After	Before	After
250	20		16	
500		3	25	1
750	30			
1,000	45	6	62	7
2,000		10		7
3,000		10		16

Example 2.—Compositions

(A) To a solution of 100 mg. of tyramine base (as the hydrochloride) in 10 cc. of isotonic sodium chloride is added a mixture of 0.15 mg. of methyl p-hydroxy benzoate and 0.2 mg. of propyl p-hydroxybenzoate. The solution is sterilized by filtration and diluted to 100 ml. with sterile isotonic sodium chloride solution. It is then used to fill sterile 1 cc. and 2 cc. ampules which are then sealed under sterile conditions.

(B) The composition of part A is prepared, using the citrate, sulfate, hydrobromide, and phosphate of tyramine.

(C) The composition of part A is prepared, using 0.9 mg. of benzylalcohol or 0.5 mg. of phenol in place of the mixture of p-hydroxybenzoates.

(D) The composition of part A is prepared without the p-hydroxybenzoate preservative mixture. Upon being sealed into the ampules, the solution can be stored for a long period of time without deterioration.

We claim:

1. A method of diagnosing pheochromocytoma which comprises determining in a patient the pressor response in systolic blood pressure to intravenous injection of 250–1000 micrograms of a pharmaceutically acceptable salt of tyramine, based on the free base, said tyramine being in an isotonic saline solution of 500–1000 micrograms per ml. concentration, whereby a pressor response greater than 20 mm. of mercury in systolic blood pressure shows presence of said disease.

2. The method of claim 1, wherein said saline solution of tyramine also contains a pharmaceutically acceptable preservative.

3. The method of claim 2 in which said preservative is sodium metabisulfite.

4. A method of diagnosing pheochromocytoma which comprises, in combination, the steps of:

(1) determining in a patient the base-line systolic blood pressure;

(2) successively injecting intravenously to said patient a solution of a water-soluble pharmaceutically acceptable tyramine salt, said injection comprising respectively 250 micrograms, 500 micrograms and 1000 micrograms of tyramine based on the free base; and

(3) observing pressor response of greater than 20 mm. of mercury in systolic blood pressure over the said base-line blood pressure.

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